

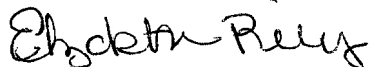
Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - *Daphnia* sp.

EPA MRID No. 47885103

Data Requirement:

EPA DP Barcode	374941
EPA MRID	47885103
EPA Guideline	850.1300

Test material:	[¹⁴ C]Cypermethrin	Radiochemical Purity:	100%
Common name	Cypermethrin		
Chemical name:	IUPAC: (RS)- α -cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate		
	CAS name: (RS)- α -cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate		
	CAS No.: 52315-07-8		
	Synonyms: none reported		

Primary Reviewer: Elizabeth A. Riley EPA, EFED, ERB6**Signature:****Date:**

4/13/11

Secondary Reviewer(s): Brian D. Keirnan EPA, EFED, ERB6**Signature:****Date:**

4/13/11

EPA PC Code 109702**Date Evaluation Completed:**

CITATION: Cafarella, M.A. 2008. Cypermethrin: Full Life Cycle Toxicity Test with Water Fleas, *Daphnia Magna* Under Flow-Through Conditions, Following OECD Guideline #211, and OPPTS Draft Guideline 850.1300. Unpublished study performed by Springborn Smithers Laboratories (Europe), Horn, Switzerland. Laboratory Study No. 1084.012.231. Study sponsored by FMC Corporation, Princeton, NJ. Study initiated June 22, 2007 and completed April 9, 2008.



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Total Length

NOAEC: 8.1 ng TRR/L

LOAEC: 18 ng TRR/L

Dry Weight

NOAEC: 4.1 ng TRR/L

LOAEC: 8.1 ng TRR/L

Endpoints affected: offspring production, total length, and dry weight

Most sensitive endpoint(s): offspring production and dry weight

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD Guideline No. 211 (1998), and
U.S. EPA OPPTS Guideline No. 850.1300 (*draft*, 1996).

1. OCSPP (form. OPPTS) guidance states that among replicate test chambers of a given level, the measured concentration of the test material should not vary more than 20%. In this study, reviewer-calculated analytical variation (high:low) of total radioactive residues (TRR) of cypermethrin was 29, 12, 44, 34, and 20% for the nominal 1.9, 3.8, 7.5, 15, and 30 ng ai/L treatment levels, respectively.
2. The analytical LOD and LOQ were not reported.
3. The reviewer's analysis detected a slight (4%), but significant ($p < 0.05$) reduction in body length of solvent control daphnia, compared to negative control daphnia. US EPA guidance on the use of solvents maintains that if a solvent is used, it should not interfere with the test material or organism response. Given the magnitude of the reduction and effect on only one endpoint, the reviewer does not suspect that solvent interference was a factor in this study.

These deviations do not affect the scientific soundness of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. This study was conducted in accordance with the Swiss Ordinance relating to Good Laboratory Practice and Compliance Monitoring (1997) with the following exceptions: routine water and food contaminant screening analyses for pesticides, PCBs, and metals.

A. MATERIALS:

1. Test Material [¹⁴C]Cypermethrin

Description: Solution in toluene

Lot No./Batch No. : CFQ13998 – Batch 1

Radiochemical Purity: 100%

Specific Activity: 55 mCi/mmol

Stability of compound under test conditions:

In addition to LSC analyses, concentrations of parent [¹⁴C]cypermethrin were determined in the highest treatment level (i.e., nominal 30 ng ai/L) using thin-layer chromatography on Days 0, 7, 13, and 21. Results indicated that

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cypermethrin was stable throughout the study, accounting for 89 to 94% of the recovered radioactivity.

**Storage conditions of
test chemicals:** -20°C

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Physicochemical properties of cypermethrin.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test Organism:

Species: *Daphnia magna*, first instar, <24 hours old
EPA and OECD recommend *Daphnia magna*

Age of the parental stock: Not reported
EPA recommends that young daphnids ≤24 hours old from a separate parental culture be used

Source: Laboratory culture
EPA requires all test organisms must be produced from laboratory reared culture that has been maintained for at least 21 days at test conditions in dilution water with renewal of the culture medium at least three times per week.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A 16-day exploratory range-finding study was performed under flow-through conditions by exposing daphnid neonates (<24 hours old) to nominal concentrations of 0 (negative and solvent controls), 10, 26, 64, 160, and 400 ng ai/L. There were two replicates per level, with 10 daphnia per replicate vessel. After 16 days of exposure, survival averaged 75, 75, 90, 20, 10, 0, and 0% in the negative control, solvent control, 10, 26, 64, 160, and 400 ng ai/L levels, respectively. The number of offspring per parent averaged 44, 44, 42, 34, and 7.0 and terminal lengths averaged 3.72, 3.66, 3.77, 3.36, and 2.82 mm for the negative control, solvent control, 10, 26, and 64 ng ai/L treatment levels, respectively. Nominal concentrations selected for use in the definitive study were based upon these results and consultation with the Sponsor.

b. Definitive Study

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Table 1: Experimental Parameters

Parameter	Details	Remarks
		<i>Criteria</i>
<u>Parental acclimation:</u> Period: Conditions: (same as test or not) Feeding: Health (any mortality observed):	Continuous Same as test. Daily with 0.5 to 3.0 mL of a solution containing <i>ca.</i> 4 x 10 ⁷ cells/mL of the unicellular green algae, <i>Ankistrodesmus falcatus</i> . Healthy: no signs of stress such as high mortality, presence of males, or ephippia.	Brood daphnia were maintained in dilution water at 17.9 to 21.5°C under a 16-hour light photoperiod. The culture water (modified Elendt M4 medium) had a total hardness of 150 to 156 mg/L as CaCO ₃ , alkalinity of 25 to 26 mg/L as CaCO ₃ , pH of 7.60 to 7.76, and a specific conductance of 395 to 400 µS/cm. <i>EPA recommends that prior to testing, daphnids that are at least 10-12 days old (those that have had at least one brood) should be separated from the culture, put in separate container and maintained for at least 21 days to insure that good health conditions are present</i>
<u>Test condition:</u> static renewal/flow-through: Type of dilution system- for flow through method. Renewal rate for static renewal	Flow-through Modified proportional diluter N/A	There were 6.43 volume replacements per day per test vessel. <i>(EPA requires consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)</i>
Aeration, if any	None reported.	<i>EPA recommends test chambers should not be aerated</i>

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Parameter	Details	Remarks
		Criteria
Duration of the test	21 days	<i>Recommended duration is 21 days.</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size (for growth and reproduction/survival test): Fill volume:	Glass jars with drain holes located <i>ca.</i> 15-cm from the bottom. The drain holes were covered with Nitex® 400-mesh screen. 1.6 L 1.4 L	1. <u>Recommended Material:</u> Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <u>Recommended Size:</u> 250 ml with 200 ml fill volume; 100 ml with 80 ml fill volume OECD guideline recommends that parent animals be maintained individually; one per vessel, with 50 - 100 ml of medium in each vessel.
Source of dilution water	Modified Elendt M4 medium prepared in a 4000-L polypropylene resin tank. The dilution water was aerated and allowed to equilibrate for 1 day prior to use. Water quality was determined following preparation of each batch: total hardness ranged from 152 to 162 mg/L as CaCO ₃ , alkalinity ranged from 26 to 37 mg/L as CaCO ₃ , pH ranged from 7.36 to 7.70, and specific conductance ranged from 360 to 420 µS/cm.	Representative samples of the dilution water were periodically analyzed for pesticides, PCBs, and toxic metals. It was reported that none of the compounds were detected at concentrations that were considered toxic in any of the water samples analyzed in agreement with ASTM guidelines (2002). <i>Recommended source of dilution water includes unpolluted well or spring water that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).</i>

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Parameter	Details	Remarks
		<i>Criteria</i>
<u>Number of organisms:</u> For growth and reproduction: For survival test:	10 organisms per replicate Same	Loading did not cause the DO to fall to <60% ASV. <i>Recommended number of organisms include 22 daphnids/test concentration; 7 test chambers should contain 1 daphnid each, and 3 test chambers contain 5 daphnids each. OECD recommends holding a minimum of 10 daphnids individually for static tests. For flow-through tests, 40 animals should be divided into 4 groups of 10 animals at each test concentration.</i>
<u>Treatment Concentrations:</u> nominal: mean-measured:	0 (negative and solvent controls), 1.9, 3.8, 7.5, 15, and 30 ng ai/L <LOQ (controls), 1.2, 2.4, 4.1, 8.1, and 18 ng TRR/L	Water samples were collected from two replicates per level (alternating) at 0, 7, 13, and 21 Days. Due to low recoveries at the 7.5-ng ai/L level on Day 13, an additional sample was collected on Day 14. Samples were analyzed for total radioactive residues of cypermethrin using LSC. In addition, aliquots from the highest treatment level were analyzed for parent cypermethrin using TLC (see Reviewer's Comments section). A high level of analytical variation was indicated for all except the 3.8 ng ai/L level. Based upon LSC results, analytical variation (reviewer-calculated) was 29, 12, 44, 34, and 20% for the nominal 1.9, 3.8, 7.5, 15, and 30 ng ai/L levels, respectively. Mean-measured concentrations ranged from 54 to 65% of nominal concentrations.

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Parameter	Details	Remarks
		<i>Criteria</i>
Solvent (type, percentage, if used)	Acetone, 50 µL/L.	<i>Solvent concentration should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone and ethanol. OECD recommends #0.1 ml/L of solvent.</i>
Lighting	16-hours light, 8-hours dark; light intensity at the test solutions' surface ranged from 235 to 410 Lux.	<i>Recommended photoperiod is 16 hours light and 8 hours of dark.</i>
Recovery of chemical: Frequency of measurement: LOD: LOQ:	70 to 96% of nominal (excluding one outlier of 65%) Days 0, 7, 13/14, and 21 Not reported Not reported	Based on QC samples analyzed concurrently with the test samples (total of 21 samples).
Positive control {if used, indicate the chemical and concentrations}	None tested	
Other parameters, if any Feeding:	During the test, daphnia were fed three times daily with 3.0 mL of algal suspension (<i>Ankistrodesmus falcatus</i> , 4 x 10 ⁷ cells/mL).	

2. Observations:

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Table 2: Observations

Parameters	Details	Remarks
		Criteria
Data endpoints measured (list)	<ul style="list-style-type: none"> - Parental immobility (mortality) - Other parental sub-lethal effects - Time to first brood release - Offspring production - Offspring immobility - Terminal length and dry weight of surviving P-generation 	<p><i>Recommended endpoints measured:</i></p> <ul style="list-style-type: none"> - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs.
Observation intervals	All test vessels were examined daily. Assessments of offspring released were determined beginning on Day 10 and three times per week thereafter. Growth measurements were determined on Day 21.	
Were raw data included?	Yes	
Other observations, if any	The physical characteristics of the test solutions were recorded daily.	

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II. RESULTS AND DISCUSSION

A. MORTALITY:

No statistically-significant differences were observed in survival, although survival at the highest treatment level was notably lower than all other levels and controls. After 21 days, survival averaged 97.5, 95.0, 95.0, 95.0, 97.5, 100, and 60.0% for the (mean-measured) negative control, solvent control, 1.2, 2.4, 4.1, 8.1, and 18 ng total radioactive residues (TRR)/L treatment levels, respectively. The 21-day EC₅₀ (with 95% C.I.) was reported by the study author to be 43 (9.0 to not calculable) ng TRR/L, and the NOAEC was reported to be 18 ng TRR/L.

Table 3: Effect of Cypermethrin on Survival, Reproduction, and Growth of *Daphnia* sp.

Treatment Mean-measured (and nominal) conc. ng TRR/L ^(a)	Cumulative Mortality (dead or immobile)		Mean Day of First Brood	Mean No. Young Per Adult	Mean Total Body Length, mm ± SD	Mean Dry Weight, mg ± SD
	No.	%				
Control (dilution water only)	1	2.5	Not reported	75	4.14 ± 0.16	0.66 ± 0.20
Solvent control	2	5.0	Not reported	65	3.97 ± 0.17	0.53 ± 0.16
1.2 (1.9)	2	5.0	Not reported	68	4.15 ± 0.14	0.59 ± 0.20
2.4 (3.8)	2	5.0	Not reported	76	4.07 ± 0.15	0.55 ± 0.15
4.1 (7.5)	1	2.5	Not reported	66	4.09 ± 0.16	0.67 ± 0.14
8.1 (15)	0	0	Not reported	55*	4.11 ± 0.17	0.49 ± 0.16*
18 (30)	16	40	Not reported	41*	3.83 ± 0.20*	0.44 ± 0.14*
NOAEC, ng TRR/L	18		4.1		8.1	4.1
LOAEC, ng TRR/L	>18		8.1		18	8.1
LC/EC ₅₀ (with 95% C.I.), mg ai/L	43 (9.0-not calc.) ^(b)		Not reported	26 (14 to 138)	Not reported	Not reported

^(a) For comparison purposes, reviewer-calculated time-weighted average (TWA) concentrations were 1.2, 2.4, 4.2, and 8.4 ng TRR/L, respectively.

^(b) The EC₅₀ was extrapolated; thus, robust confidence intervals could not be calculated.

*Statistically-reduced compared to the negative control (p<0.05).

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B. EFFECTS ON REPRODUCTION AND GROWTH:

It was reported that both control groups released their first brood of offspring on Day 9 (although a quantitative assessment was not made until Day 10). First brood release for all treatment levels ranged from Days 9 to 12, which was reported as consistent with the negative and solvent control performance. Actual days to first brood release were not calculated, and could not be determined by the reviewer as offspring were not counted daily during the reproductive period.

The cumulative number of offspring released per female averaged 75 for the negative control, 65 for the solvent control, and 68, 67, 66, 55, and 41 for the mean-measured 1.2, 2.4, 4.1, 8.1, and 18 ng TRR/L treatment levels, respectively. Differences were statistically-reduced ($p < 0.05$) compared to the negative control at the 8.1 and 18 ng TRR/L levels. The EC_{50} (with 95% C.I.) was reported to be 26 (14 to 138) ng TRR/L, and the NOAEC was reportedly 4.1 ng TRR/L.

There were no immobilized offspring produced.

At study termination, mean total body lengths of surviving daphnia were 4.14, 3.97, 4.15, 4.07, 4.09, 4.11, and 3.83 mm for the negative control, solvent control, 1.2, 2.4, 4.1, 8.1, and 18 ng TRR/L levels, respectively. The differences was statistically-reduced ($p < 0.05$) compared to the negative control at the 18 ng TRR/L level. Mean dry weight of surviving first-generation daphnia was 0.66, 0.53, 0.59, 0.55, 0.67, 0.49, and 0.44 mg, respectively. Dry weights were statistically-reduced ($p < 0.05$) compared to the negative control at the 8.1 and 18 ng TRR/L levels. Based on terminal dry weight, the NOAEC for growth was reported to be 4.1 ng TRR/L.

C. REPORTED STATISTICS:

Data that were statistically analyzed included organism survival (i.e., immobilization), reproduction (cumulative number of offspring produced per female), and growth (as total body length and dry body weight). The time to first brood release data were not statistically assessed.

A t-Test was used to compare the performance of the dilution water control with that of the solvent control. A statistically-significant difference was observed between the two control groups for terminal length. Since further statistical analyses indicated that this endpoint was not the most sensitive, this difference in control performance had no notable effect on the outcome of the study. All treatment levels were compared to the performance of the negative control.

For all endpoints, data were assessed for normality using the Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's test. Survival data failed both assumptions, and were arc-sine transformed prior to analysis using the non-parametric Steel's Many-One Rank Test. Length data were not normally distributed and were therefore also analyzed using Steel's Many-One Rank Test. Reproductive and dry weight data met both assumptions and were evaluated with Williams' Test to establish treatment-related effects.

The NOAEC and LOAEC were based on significance data. All analyses were performed using TOXSTAT® version 3.5 and mean-measured concentrations of total radioactive residues (TRR) of cypermethrin.

The 21-day EC_{50} values (with 95% C.I.) were determined using the bionomial method.

Parental Survival

EC_{50} : 43 ng TRR/L

95% C.I.: 9.0 to not calculable

NOAEC: 18 ng TRR/L

LOAEC: >18 ng TRR/L

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Reproduction (offspring/parent)

EC₅₀: 26 ng TRR/L

95% C.I.: 14 to 138 ng TRR/L

NOAEC: 4.1 ng TRR/L

LOAEC: 8.1 ng TRR/L

Total Length

NOAEC: 8.1 ng TRR/L

LOAEC: 18 ng TRR/L

Dry Weight

NOAEC: 4.1 ng TRR/L

LOAEC: 8.1 ng TRR/L

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer verified results for survival, reproduction, length, and dry weight. The negative and solvent control data were compared for all endpoints using a Student's t-test. A significant difference ($p < 0.05$) was detected for the length endpoint only and the magnitude of the effect was small (4%), so the reviewer did not consider this to be a case of solvent interference. Data were further tested to determine if they satisfied the assumptions of normality using Shapiro-Wilk's test and homogeneity of variances using Levene's test. The data for survival and length failed to meet these assumptions, so the NOAEC and LOAEC for these endpoints were determined using the non-parametric Steele's many-one rank test. Data for reproduction and dry weight satisfied these assumptions, so the NOAEC and LOAEC were determined using ANOVA, followed by Dunnett's or William's (dose-dependent response) multiple comparison test. These analyses were conducted using the Toxstat 3.5 statistical program. The reviewer additionally used the Probit method to estimate the 21-day LC₅₀ using Toxanal 2009 and the 21-day EC₅₀ for reproduction using Nuthatch statistical software. Both values exceeded the highest levels tested and the LC₅₀ was not associated with a calculable 95% confidence interval, so these results should be interpreted with caution.

Parental Survival

EC₅₀: 49 ng TRR/L

95% C.I.: Not calculable

Slope: 1.39 (-1.20 to 3.98)

NOAEC: 18 ng TRR/L

LOAEC: >18 ng TRR/L

Reproduction (offspring/parent)

EC₅₀: 23 ng TRR/L

95% C.I.: 16 to 32 ng TRR/L

Slope: 1.56±0.369

NOAEC: 4.1 ng TRR/L

LOAEC: 8.1 ng TRR/L

Total Length

NOAEC: 8.1 ng TRR/L

LOAEC: 18 ng TRR/L

Dry Weight

NOAEC: 4.1 ng TRR/L

LOAEC: 8.1 ng TRR/L

Endpoints affected: offspring production, total length, and dry weight

Most sensitive endpoint(s): offspring production and dry weight

E. STUDY DEFICIENCIES:

Based upon results obtained from LSC analyses (total radioactivity), a high level of analytical variation was observed at all except the nominal 3.8 ng ai/L level. Reviewer-calculated percent variation was 29, 44, 34, and 20% for the nominal 1.9, 7.5, 15, and 30 ng ai/L levels, respectively. The study author reported that given the nature of the test material (which is known to be highly sorptive) and given the dynamics of the test system (e.g., presence of algae and increasing biomass), that it was not unexpected to see variable recoveries, especially at the low levels tested. Despite high analytical variation there was no overlap between treatment levels. In addition to the high analytical variation observed during the study, the analytical LOD and/or LOQ were not reported.

F. REVIEWER'S COMMENTS:

The reviewer's results agreed with the results obtained by the study author. Neither the reviewer nor the study author detected a significant reduction in survival at the highest level (because one replicate from this level overlapped with the control response and zero variance in one group required use of a non-parametric test to determine the NOAEC), but it should be noted that this endpoint was reduced an average of 40%, relative to the control.

In general, validity requirements were fulfilled. Specifically, negative and solvent control immobility was 2.5 and 5.0%, respectively (no more than 20% permissible); and each negative and solvent control daphnid living the full 21 days produced an average of 75 and 65 young, respectively (minimum of 60 required). It was not reported if any ephippia were produced by control animals.

For comparison purposes, TWA concentrations were calculated by the reviewer (refer to associated Excel worksheet in Appendix II). As TWA concentrations were comparable to mean-measured concentrations provided by the reviewer, they were not otherwise reported in the DER. TWA concentrations were calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C TWA is the time-weighted average concentration,

C j is the concentration measured at time interval j (j = 0, 1, 2,...n)

t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., t 0 = 0 hours (test initiation), t 1 =24 hours, t 2 =96 hours).

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A [¹⁴C]cypermethrin super stock solution was prepared 16 days prior to study initiation and during the study using the following purification method: 12 g of Cilicagel and 20 mL trichloroethylene (TCE) were added to a small beaker and sonicated for 5 minutes. The gel was transferred to a 30-cm column and rinsed with additional TCE. A 65-μL of [¹⁴C]cypermethrin was applied to the top of the gel column, and the column was rinsed 70 times with 6-mL fractions of TCE. Fractions were collected in 20-mL LSC vials, and LSC counts were performed on every other vial through vial no. 59. The test material eluted between fractions 21 and 57. Radiochemical purity was determined using TLC analysis from fractions 24 to 45. All fractions from 21 through 57 were pooled and evaporated to *ca.* 5 mL, then taken to dryness with nitrogen. The test material was re-dissolved in 5 mL acetone and final LSC and TLC measurements were taken. Test stock solutions were prepared prior to and during the test by diluting the appropriate amount of super stock to 50 mL with acetone. The concentrations of the stock solutions were verified using LSC prior to use.

During the study, 1000-mL water samples were extracted twice with 200 mL hexane. The organic phase was concentrated to near dryness using rotary evaporation (at 30°C and 250 mbar), then to dryness under nitrogen. The residues were dissolved in 1 mL acetonitrile, and aliquots were analyzed for total radioactive residues (TRR) of cypermethrin using LSC. Samples from the highest treatment level (i.e., 30 ng ai/L) were also analyzed for parent [¹⁴C]cypermethrin using TLC (not further described). Analytical LOD and LOQ were not reported.

The experimental phase of the definitive study was conducted from January 25 to February 15, 2008.

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G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. Based upon treatment-related reductions in reproduction (offspring per female) and dry weights of surviving first-generation daphnia (the most sensitive endpoints), the NOAEC and LOAEC were 4.1 and 8.1 ng TRR/L, respectively. The 21-day EC₅₀ (with 95% C.I.) for offspring production was 23 (16 to 32) ng TRR/L. The total lengths of daphnia were also reduced at the 18 ng TRR/L treatment level. No statistically-significant effects on survival of first-generation daphnia were observed. The 21-day EC₅₀ (with 95% C.I.) for immobility was 49 (95% C.I. not calculable) ng TRR/L.

Parental Survival

EC₅₀: 49 ng TRR/L

95% C.I.: Not calculable

Slope: 1.39 (-1.20 to 3.98)

NOAEC: 18 ng TRR/L

LOAEC: >18 ng TRR/L

Reproduction (offspring/parent)

EC₅₀: 23 ng TRR/L

95% C.I.: 16 to 32 ng TRR/L

Slope: 1.56±0.369

NOAEC: 4.1 ng TRR/L

LOAEC: 8.1 ng TRR/L

Total Length

NOAEC: 8.1 ng TRR/L

LOAEC: 18 ng TRR/L

Dry Weight

NOAEC: 4.1 ng TRR/L

LOAEC: 8.1 ng TRR/L

Endpoints affected: offspring production, total length, and dry weight

Most sensitive endpoint(s): offspring production and dry weight

III. REFERENCES:

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- ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
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- OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and management Committee. ENV/MC/CHEM (98)17. OECD Paris. France. 41 pp.

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Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

EPA MRID No. 47885103

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Title: Adult survival

File: 5103s Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean = 95.0000 Calculated t value = 0.0000
GRP2 (Blank cntl) Mean = 95.0000 Degrees of freedom = 6
Difference in means = 0.0000

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Adult survival

File: 5103s Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Neg Control	95.0000				
2	1.2	95.0000	18.00	10.00	4.00	
3	2.4	97.5000	20.00	10.00	4.00	
4	4.1	97.5000	20.00	10.00	4.00	
5	8.1	100.0000	22.00	10.00	4.00	
6	18	60.0000	11.00	10.00	4.00	

Critical values are 1 tailed (k = 5)

Title: Cumulative progeny/female

File: 5103r Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean = 74.5000 Calculated t value = 0.9283
GRP2 (Blank cntl) Mean = 65.5000 Degrees of freedom = 6
Difference in means = 9.0000

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Cumulative progeny/female

File: 5103r Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

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D = 860.7500
W = 0.9795

Critical W = 0.8840 (alpha = 0.01 , N = 24)
W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Cumulative progeny/female

File: 5103r

Transform:

NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	53.2083	10.6417	0.5139
Within (Error)	18	372.7500	20.7083	
Total	23	425.9583		

(p-value = 0.7623)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Cumulative progeny/female

File: 5103r

Transform:

NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	2928.2083	585.6417	12.2469
Within (Error)	18	860.7500	47.8194	
Total	23	3788.9583		

(p-value = 0.0000)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

EPA MRID No. 47885103

Since $F > \text{Critical } F$ REJECT H_0 : All equal ($\alpha = 0.05$)

Title: Cumulative progeny/female

File: 5103r

Transform:

NO TRANSFORMATION

Dunnett's Test

- TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
0.05					
1	Neg Control	74.5000	74.5000		
2	1.2	68.0000	68.0000	1.3293	
3	2.4	66.5000	66.5000	1.6361	
4	4.1	65.5000	65.5000	1.8406	
5	8.1	55.2500	55.2500	3.9368	*
6	18	40.5000	40.5000	6.9533	*

Dunnett critical value = 2.4100 (1 Tailed, $\alpha = 0.05$, $df = 5, 18$)

Title: Cumulative progeny/female

File: 5103r

Transform:

NO TRANSFORMATION

Dunnett's Test

- TABLE 2 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.2	4	11.7843	15.8	6.5000
3	2.4	4	11.7843	15.8	8.0000
4	4.1	4	11.7843	15.8	9.0000
5	8.1	4	11.7843	15.8	19.2500
6	18	4	11.7843	15.8	34.0000

Title: Cumulative progeny/female

File: 5103r

Transform:

NO TRANSFORMATION

William's Test

- TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	4	74.5000	74.5000	74.5000
2	1.2	4	68.0000	68.0000	68.0000
3	2.4	4	66.5000	66.5000	66.5000

Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

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4	4.1	4	65.5000	65.5000	65.5000
5	8.1	4	55.2500	55.2500	55.2500
6	18	4	40.5000	40.5000	40.5000

Title: Cumulative progeny/female

File: 5103r

Transform:

NO TRANSFORMATION

William's Test - TABLE 2 OF 2

Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	74.5000				
1.2	68.0000	1.3293		1.7300	k= 1, v=18
2.4	66.5000	1.6361		1.8200	k= 2, v=18
4.1	65.5000	1.8406		1.8500	k= 3, v=18
8.1	55.2500	3.9368	*	1.8600	k= 4, v=18
18	40.5000	6.9533	*	1.8700	k= 5, v=18

s = 6.9152

Title: Total length

File: 5103l

Transform:

NO TRANSFORMATION

t-Test of Solvent and Blank Controls

Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean = 4.1450 Calculated t value = 2.6715

GRP2 (Blank cntl) Mean = 3.9675 Degrees of freedom = 6

Difference in means = 0.1775

2-sided t value (0.05, 6) = 2.4469** Significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Total length

File: 5103l

Transform:

NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.2073

W = 0.8808

Critical W = 0.8840 (alpha = 0.01 , N = 24)

W = 0.9160 (alpha = 0.05 , N = 24)

Data FAIL normality test (alpha = 0.01). Try another transformation.

Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

EPA MRID No. 47885103

Warning - The first three homogeneity tests are sensitive to non-normality and should not be performed with this data as is.

Title: Total length

File: 51031

Transform:

NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0453	0.0091	2.2565
Within (Error)	18	0.0722	0.0040	
Total	23	0.1175		

(p-value = 0.0928)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Total length

File: 51031

Transform:

NO TRANSFORMATION

Steel's Many-One Rank Test

Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Neg Control	4.1450				
2	1.2	4.1475	17.50	10.00	4.00	
3	2.4	4.0700	13.00	10.00	4.00	
4	4.1	4.0925	14.50	10.00	4.00	
5	8.1	4.1100	16.00	10.00	4.00	
6	18	3.7525	10.00	10.00	4.00	*

Critical values are 1 tailed (k = 5)

Title: Dry weight

File: 5103w

Transform:

NO TRANSFORMATION

t-Test of Solvent and Blank Controls

Ho: GRP1 Mean = GRP2 Mean

Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

EPA MRID No. 47885103

GRP1 (Solvent cntl) Mean = 0.6625 Calculated t value = 1.7241
GRP2 (Blank cntl) Mean = 0.5300 Degrees of freedom = 6
Difference in means = 0.1325

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dry weight

File: 5103w

Transform:

NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.1096

W = 0.9593

Critical W = 0.8840 (alpha = 0.01 , N = 24)

W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dry weight

File: 5103w

Transform:

NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0092	0.0018	0.7081
Within (Error)	18	0.0466	0.0026	
Total	23	0.0558		

(p-value = 0.6250)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)

= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dry weight

File: 5103w

Transform:

NO TRANSFORMATION

ANOVA Table

Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

EPA MRID No. 47885103

SOURCE	DF	SS	MS	F
Between	5	0.1905	0.0381	6.2571
Within (Error)	18	0.1096	0.0061	
Total	23	0.3002		

(p-value = 0.0016)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Dry weight

File: 5103w

Transform:

NO TRANSFORMATION

Dunnett's Test

- TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
0.05					
1	Neg Control	0.6625	0.6625		
2	1.2	0.5925	0.5925	1.2685	
3	2.4	0.5500	0.5500	2.0387	
4	4.1	0.6675	0.6675	-0.0906	
5	8.1	0.4900	0.4900	3.1260	*
6	18	0.4200	0.4200	4.3945	*

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dry weight

File: 5103w

Transform:

NO TRANSFORMATION

Dunnett's Test

- TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	4			
2	1.2	4	0.1330	20.1	0.0700
3	2.4	4	0.1330	20.1	0.1125
4	4.1	4	0.1330	20.1	-0.0050
5	8.1	4	0.1330	20.1	0.1725
6	18	4	0.1330	20.1	0.2425

Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

EPA MRID No. 47885103

Title: Dry weight
File: 5103w Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

			ORIGINAL	TRANSFORMED	ISOTONIZED
GROUP	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	Neg Control	4	0.6625	0.6625	0.6625
2	1.2	4	0.5925	0.5925	0.6033
3	2.4	4	0.5500	0.5500	0.6033
4	4.1	4	0.6675	0.6675	0.6033
5	8.1	4	0.4900	0.4900	0.4900
6	18	4	0.4200	0.4200	0.4200

Title: Dry weight
File: 5103w Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	0.6625				
1.2	0.6033	1.0722		1.7300	k= 1, v=18
2.4	0.6033	1.0722		1.8200	k= 2, v=18
4.1	0.6033	1.0722		1.8500	k= 3, v=18
8.1	0.4900	3.1260	*	1.8600	k= 4, v=18
18	0.4200	4.3945	*	1.8700	k= 5, v=18

s = 0.0780

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
18	40	16	40	0
8.1	40	0	0	0
4.1	40	1	2.5	0
2.4	40	2	5	0
1.2	40	2	5	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 0

Data Evaluation Record on the Chronic Toxicity of [¹⁴C]Cypermethrin to Freshwater Invertebrates - Daphnia sp.

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THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET
BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT
BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND
100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	3.473185	5.193116	1.383066E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.388143
95 PERCENT CONFIDENCE LIMITS = -1.198867 AND 3.975152

INTERCEPT = -2.345617

LC50 = 48.94998
95 PERCENT CONFIDENCE LIMITS = 9.643511 AND +INFINITY

LC25 = 15.99011
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 5.841232
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC05 = 3.197333
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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APPENDIX II: COPY OF REVIEWER'S TWA CALCULATIONS (USING EXCEL):

Time-Weighted
Concentrations

Nominal Concentration (ng ai/L)	Time (Day)	Measured Concentration (ng TRR/L)	TWA (ng TRR/L)
1.9	0	1.2	
	7	1.4	
	13	1	
	21	1.3	
		TWA	1.2143
		% variation (high:low)	29
3.8	0	2.3	
	7	2.3	
	13	2.6	
	21	2.4	
		TWA	2.4190
		% variation (high:low)	12
7.5	0	2.9	
	7	3.7	
	14	5.2	
	21	4.7	
		TWA	4.2333
		% variation (high:low)	44
15	0	6.5	
	7	7.5	
	13	9.9	
	21	8.7	
		TWA	8.3619
		% variation (high:low)	34
30	0	18	
	7	17	
	13	16	
	21	20	
		TWA	17.4048
		% variation (high:low)	20